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(19)

(54) VACCINE PREPARATIONS CONTAINING ADJUVANTS

(71) We, RECHERCHE ET INDUSTRIE THÉRAPEUTIQUES, R.I.T., a Belgian Body Corporate of 13, rue du Tilleul, B—1320 Genval, Belgium, do hereby declare this invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

In our Specification Serial No. 1295666 there is described the use of oxidized polysaccharides as anti-infectious agents.

It has now been found that, independently of their anti-infectious activity, these oxidized polysaccharides are also valuable adjuvants for vaccines.

Thus, the present invention relates to pharmaceutical compositions as described in Specification Serial No. 1295666 which are vaccine preparations with adjuvant comprising as adjuvant and per dosage unit of vaccine from 1 to 200 mg. of an oxidized polysaccharide having at least 50% of the monosaccharide rings open, substantially all the open rings oxidized to the carboxylic acid state and substantially all the C—O—C linkages originally present in the original polysaccharide still intact. Preferably, the said oxidized polysaccharide is present in the preparation as the free acid form or in the form of a physiologically acceptable alkaline salt. Preferably also, the molecular weight of the oxidized polysaccharide is at least 5,000.

The present invention also relates to a process for enhancing the antigenic potency of a vaccine, said process comprising adding thereto as an adjuvant and per dosage unit of vaccine from 1 to 200 mg. of an oxidized polysaccharide having at least 50% of the monosaccharide rings open, substantially all the open rings oxidized to the carboxylic acid state and substantially all the C—O—C linkages originally present in the original polysaccharide still intact. In said process, the oxidized polysaccharide is used under the free acid form or under the form of a physiologically acceptable alkaline salt. Preferably also, the molecular weight of the used oxidized polysaccharide is at least 5,000.

[*Price 25p*]

The oxidized polysaccharides which, according to the present invention, are vaccine adjuvants and the preparation thereof are fully described in our Specification Serial No. 1295666.

Substantially, the polysaccharides are amylose, amylopectin, dextran, polygalacturonic acid, alginic acid, cellulose and guaran.

It has now been found that said oxidized polysaccharides do substantially enhance the antigenic potency of the vaccines to which they are added.

The preferred adjuvant of this invention is a linear condensation copolymer containing 1,4-linked anhydro-α-D-glucopyranose units and 1,4-linked anhydro - 1,4 - dihydroxy - 1,4-dicarboxy - 3 - hydroxymethyl - 2 - oxabutane units (COAM). It is obtained by oxidation of amylose with sodium metaperiodate and then with sodium chlorite. It has been found to be a highly effective vaccine adjuvant.

The vaccine adjuvant effect of COAM has been demonstrated as follows: Groups of 12 mice were injected intraperitoneally with one of the following formulations: influenza vaccine (A_2 Hongkong/1/1968 or B/Neth strains), vaccine plus COAM, vaccine plus incomplete Freund adjuvant (IFA), vaccine plus polyinosinic-polycytidylic acid (poly I—C), vaccine plus IFA and poly I—C, and vaccine plus IFA and COAM. The dosages were 0.0625 ml. of vaccine per mouse, 25 μg of poly I—C per mouse, 4 mg. of COAM per mouse, and 0.125 ml. of IFA per mouse. The experiment was done on 3 sets of mice which were bled at 7, 14 and 28 days post-injection. The mice were bled at the orbital sinus and blood was immediately diluted 1:5 in phosphate buffered saline (NaCl 8 g, KCl 0,20 g, Na_2HPO_4 12 aq 2,89 g, KH_2PO_4 0,2 g, pyrogen free distilled water up to 1 l.). Antibody titers were determined on individual samples using the hemagglutination-inhibition (HA—I) test. The vaccine contained 10,500 HA units/ml. of A_2 Hk/1/68 strain or 7,500 HA units/ml. of B/Neth strain.

The results obtained are presented below in Tables I and II

TABLE I

Average HA—I—Titer of Mice Treated with Vaccine (A, Hk Strain), COAM, Poly I—C and IFA *

Treatment	Titer		
	7 days	14 days	21 days
vaccine only	<10	<10	<10
vaccine + poly I—C	<10	16	20
vaccine + COAM	<44	66	200
vaccine + IFA	<10	26	63
vaccine + IFA + poly I—C	<10	40	220
vaccine + IFA + COAM	32	90	350

* Serum samples were assayed individually. Values shown in the table are reciprocals of geometric means of end-point dilutions.

TABLE II

Average HA—I—Titer of Mice Treated with Vaccine (B/Neth Strain), COAM, Poly I—C and IFA*

Treatment	Titer		
	7 days	14 days	21 days
vaccine only	50	71	25
vaccine + poly I—C	80	160	32
vaccine + COAM	320	550	100
vaccine + IFA	160	200	80
vaccine + IFA + poly I—C	220	370	210
vaccine + IFA + COAM	210	500	280

* Serum samples were assayed individually. Values shown in the table are reciprocals of geometric means of end-point dilutions.

The results indicate that at a dosage of 160 mg/kg in mice, COAM, either alone or in combination with IFA, produced antibody titers considerably higher than with the vaccine alone. It was also superior to IFA or poly I—C.

Further studies on enhancement of influenza vaccine were done on guinea pigs. In a first experiment it was determined that an adjuvant effect was obtained after intramuscular as well as after intraperitoneal injection. The effect of dose of COAM was also studied: one group of guinea pigs received the same formulation as the mice, i.e. 4 mg. of COAM per dose of vaccine. A second group was given the same dose of COAM as mice but calculated on a weight basis, i.e. 40 mg. per guinea pig. It was reasoned that this would give an indica-

tion as to whether adjuvant activity was due to local or systemic effects. Groups of 10 guinea pigs were used. They were given 0.5 ml. of vaccine containing A equi/1 antigen. Antibodies were determined at 3, 6, 9 and 12 weeks after vaccination. Average values are summarized in Table III.

TABLE III

Adjuvant Effect of COAM on Antibody Induction by Influenza Vaccine in Guinea Pigs

Vaccination schedule *	Antibody titers **			
	3 weeks	6 weeks	9 weeks	12 weeks
Intramuscular vaccination				
— Vaccine only	96	80	85	30
— Vaccine + COAM 4 mg.	216	108	112	60
— Vaccine + COAM 40 mg.	368	270	182	112
Intraperitoneal vaccination				
— Vaccine only	120	158	91	28
— Vaccine + COAM 4 mg.	209	138	98	37
— Vaccine + COAM 40 mg.	446	364	295	98

* Formulations were such that the animals received 0.5 ml. of A equi/1—vaccine mixed with 0.5 ml. of either saline or COAM at the indicated doses.

**Serum samples were assayed individually. Values shown in the Table are reciprocals of geometric means of end-point dilutions.

Adjuvant activity was present both with intramuscular and intraperitoneal treatments ($P < 0.001$). However, a small dose of COAM only stimulated early induction of antibody (3 weeks), as the effect waned at 6, 9 and 12 weeks (interaction between bleeding time and treatment significant at level $P < 0.001$).

The requirement for a higher dose in guinea pigs than in mice might have pointed to a systemic rather than a local mechanism. Therefore, in a last experiment, it was investigated whether COAM had adjuvant activity when injected at another site than the vaccine. Groups of 7 guinea pigs were given A equi/1 vaccine intraperitoneally or intravenously. COAM (40 mg. per dose) was included or given separately at a distant site. As Table IV shows, COAM had adjuvant activity only when given at the same injection site as the vaccine. It seems unlikely therefore that it acts by generalized stimulation of immunity mechanisms.

TABLE IV

Adjuvant Effect of COAM on Antibody Induction by Influenza Vaccine in Guinea Pigs

Vaccination schedule*	Antibody titer 3 weeks after vaccination**
Intramuscular vaccination	
— Vaccine only	52
— Vaccine + COAM 40 mg.	195
— Vaccine + COAM 40 mg. i.p.	64
Intraperitoneal vaccination	
— Vaccine only	64
— Vaccine + COAM 40 mg.	182
— Vaccine + COAM 40 mg. i.m.	80

* Formulations were such that the animals received 0.5 ml. of A equi/l — vaccine, mixed with 0.5 ml. of either saline or COAM at the indicated doses.

** Serum samples were assayed individually. Values shown in the Table are reciprocals of geometric means of end-point dilutions.

The vaccine adjuvants of this invention may be used to potentiate the effects of vaccines. They may be used with vaccines such as influenza, parainfluenza, polio, measles, mumps, hepatitis. Eastern and Western equine encephalomyelitis, feline viral rhinotracheitis, foot-and-mouth, cytomegaloviruses, feline picorna viruses, streptococcus, tetanus, diphtheria, pertussis, colibacillosis, pasteurellosis and leptospira. They may in general be incorporated in the vaccines in amounts such that, when the appropriate amount of vaccine is administered, the dosage of adjuvant will be from about 1 mg. to 200 mg. per dose of vaccine. The adjuvanted vaccines are administered by injection, preferably intramuscularly.

Adjuvants of this addition other than COAM include oxidized amylopectin (a branched condensation copolymer containing 1,4 and 1,6-linked anhydro - α - D - glucopyranose units and 1,4-linked anhydro - 1,4 - dihydroxy - 1,4 - dicarboxy - 3 - hydroxymethyl - 2 - oxabutane units), oxidized polygalacturonic acid (a linear condensation copolymer containing 1,4-linked anhydro - α - D - galacturonopyranose units and 1,4-linked anhydro - 1,4-dihydroxy - 1,3,4 - tricarboxy - 2 - oxabutane units), oxidized alginic acid (a linear condensation copolymer containing 1,4-linked anhydro-β-D-mannuronopyranose units, 1,4-linked anhydro-α-L-guluronopyranose units, and 1,4-linked anhydro - 1,4 - dihydroxy - 1,3,4 - tricarboxy - 2 - oxabutane units), oxidized cellulose (a linear condensation copolymer containing 1,4 - linked - β - D - glucopyranose units and 1,4-linked anhydro - 1,4 - dihydroxy - 1,4 - dicarboxy - 3 - hydroxymethyl - 2-oxabutane units), oxidized dextran (a condensation copolymer containing 1,6 - linked - α-D - glucopyranose units and 1,4 - linked - anhydro - 1,4 - dihydroxy - 1,3 - dicarboxy - 2-oxabutane units), and oxidized guaran (a condensation copolymer consisting of a linear chain of 1,4-linked anhydro - β - D - mannopyranose units and 1,4-linked anhydro - 1,4-dihydroxy - 1,4 - dicarboxy - 3 - hydroxymethyl - 2 - oxabutane units as substituants of half of the hydroxymethyl functions of the main chain).

The following Examples, which are not limiting, describe some vaccine preparations containing adjuvants according to the present invention.

EXAMPLE 1.

Oxidized amylose (20mg.) as obtained in example 1 of Specification Serial No. 1295666 is dissolved in 2 ml. of sterile phosphate buffered saline (NaCl 8 g, KCl 0.20 g, Na_2HPO_4 12 aq 2.89 g. KH_2PO_4 0.2 g, pyrogen free distilled water up to 1 l.)

The solution is added to a single dose of lyophilized killed influenza vaccine and the composition is administered by the intramuscular route.

EXAMPLE 2.

Oxidized amylopectin (50mg.) as obtained in example 8 of Specification Serial No. 1295666 is dissolved in 2 ml. of sterile phosphate buffered saline (NaCl 8g, KCl 0.20 g, Na_2HPO_4 12 aq 2.89 g, KH_2PO_4 0.2 g, pyrogen free distilled water up to 1 l.)

The solution is added to a single dose of lyophilized killed parainfluenza vaccine and the composition is administered by the intramuscular route.

EXAMPLE 3.

Oxidized polygalacturonic acid (sodium salt) (50 mg.) as obtained in example 9 of Specification Serial No. 1295666 is dissolved in 2 ml. of sterile phosphate buffered saline (NaCl 8 g, KCl 0.20 g, Na_2HPO_4 12 aq 2.89 g, KH_2PO_4 0.2 g, pyrogen free distilled water up to 1 l.)

The solution is added to a single dose of inactivated poliomyelitis vaccine and the composition is administered by the intramuscular route.

EXAMPLE 4.

Oxidized amylose (50 mg.) as obtained in example 1 of Specification Serial No. 1295666 is dissolved in 2 ml. of sterile phosphate buffered saline (NaCl 8 g, KCl 0.20 g, Na_2HPO_4 12 aq 2.89 g, KH_2PO_4 0.2 g, pyrogen free distilled water up to 1 l.)

The solution is added to a single dose of lyophilized killed influenza vaccine and the composition is administered by the intramuscular route.

EXAMPLE 5.

Oxidized dextran (100 mg) as obtained in example 14 of Specification Serial No. 1295666 is dissolved in 2 ml. of sterile phosphate buffered saline (NaCl 8 g, KCl 0.20 g, Na_2HPO_4 12 aq 2.89 g, KH_2PO_4 0.2 g, pyrogen free distilled water up to 1 l.)

The solution is added to a single dose of diphtheria-tetanos toxoids and the composition is administered by the intramuscular route.

WHAT WE CLAIM IS:—

1. A vaccine preparation with adjuvant comprising as adjuvant and per dosage unit of vaccine from 1 to 200 mg. of an oxidized polysaccharide having at least 50% of the monosaccharide rings open, substantially all the open rings oxidized to the carboxylic acid state and substantially all the C—O—C linkages originally present in the originally polysaccharide still intact.

2. A vaccine preparation with adjuvant according to claim 1 wherein the polysaccharide is amylose.

3. A vaccine preparation with adjuvant according to claim 1 wherein the polysaccharide is amylopectin, dextran, polygalacturonic acid, alginic acid, cellulose or guaran.

4. A vaccine preparation with adjuvant according to any of claims 1 to 3, wherein the oxidized polysaccharide is present as the free acid form or in the form of a physiologically acceptable alkaline salt.

5. A vaccine preparation with adjuvant according to any of claims 1 to 4 wherein the molecular weight of the oxidized polysaccharide is at least 5,000.

6. A vaccine preparation with adjuvant, substantially as herein before described, more particularly in the Examples.

7. A process for enhancing the antigenic potency of a vaccine, said process comprising adding thereto as an adjuvant and per dosage unit of vaccine from 1 to 200 mg. of an oxidized polysaccharide having at least 50% of the monosaccharide rings open, substantially all the open rings oxidized to the carboxylic acid state and substantially all the C—O—C linkages originally present in the original polysaccharide still intact.

8. A process according to claim 7, wherein the polysaccharide is amylose.

9. A process according to claim 7, wherein the polysaccharide is amylopectin, dextran, polygalacturonic acid, alginic acid, cellulose or guaran.

10. A process according to any of claims 7 to 9, wherein the oxidized polysaccharide is present as the free acid form or in the form of a physiologically acceptable alkaline salt.

11. A process according to any of claims 7 to 10, wherein the molecular weight of the oxidized polysaccharide is at least 5,000.

12. A process for enhancing the antigenic potency of a vaccine, substantially as herein before described, more particularly in the Examples.

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